Oxaliplatin, a Potent Inhibitor of Survivin, Enhances Paclitaxel-induced Apoptosis and Mitotic Catastrophe in Colon Cancer Cells

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Background: Clinical studies have demonstrated that oxaliplatin, a novel platinum derivative, is a potent chemotherapeutic agent, especially when combined with other reagents. The aim of the present study was to explore the mechanism of such action.

Methods: Using colon cancer cell lines, we examined changes in cell cycle, apoptosis and mitotic catastrophe induced by oxaliplatin and/or paclitaxel.

Results: Oxaliplatin at its IC50 induced apoptosis and cell cycle arrest at G2–M phase. Western blot analyses indicated that oxaliplatin decreased mitosis-commencing protein cdc2 and anti-apoptotic proteins, phospho-Bcl2 and Bcl-xl in the three colon cancer cells tested. Since cdc2 stabilizes survivin, a putative IAP (inhibitor of apoptosis) family member, through phosphorylation of Thr34, we examined the level of survivin and found a marked decrease due to oxaliplatin. This finding is of particular interest because survivin is a promising molecular target against various human cancers and a key molecule involved in both apoptosis and mitotic catastrophe. When used in combination with paclitaxel (taxol), a putative apoptosis-inducing reagent, the isobologram indicated that the taxol–oxaliplatin sequence or taxol plus oxaliplatin had synergistic or additive effects, while the oxaliplatin–taxol sequence resulted in a prominent antagonism. The taxol–oxaliplatin sequence caused marked growth inhibition of DLD1 and SW480 cells, possibly due to upregulation of apoptotic and non-apoptotic pathways, respectively. Morphological surveys indicated that the non-apoptotic process could be mitotic catastrophe.

Conclusion: Our results suggest that oxaliplatin that potently inhibited survivin may exert outstanding cytotoxic effects when combined with certain chemoreagents through enhancement of apoptosis and mitotic catastrophe.

Key words: oxaliplatin – taxol – survivin – apoptosis – mitotic catastrophe

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in western countries. Approximately 84–92% of patients with CRC are treated by surgical resection, but more than half of these patients subsequently develop disease recurrence (1). Although chemotherapy is of choice for patients with recurrent tumors, intrinsic or acquired chemoresistance is frequently encountered during chemotherapy.

The efficiency of cisplatin (CDDP) is generally low in CRC, with <20% clinical responses when used alone or in combination with 5-fluorouracil (5-FU) or etoposide (2,3). Oxaliplatin is a third-generation platinum coordination complex of the 1,2-diaminocyclohexane families, which was developed after CDDP and carboplatin (CBDCA) (4,5). Compared with other classes of platinum derivatives, oxaliplatin lacks the nephrotoxicity associated with CDDP and the myelosuppression associated with CBDCA treatments. Although oxaliplatin produces DNA cross-links similar to those of cisplatin, the National Cancer Institute (NCI) screening for susceptible cancer cell lines showed distinct clustering of oxaliplatin from other platinum compounds (6), and CDDP-resistant cells generally remain sensitive to oxaliplatin. The outstanding efficacy of oxaliplatin is considered to be due to the formation of bulkier Pt–DNA adducts between two adjacent guanines or guanine and adenine, and induction of a greater deformation of the DNA structure than CDDP-DNA adducts (7–10). However,
the actual biological behavior of oxaliplatin is mostly still unknown.

To explore the underlying mechanism of action of oxaliplatin, we examined the effects of oxaliplatin treatment on apoptosis and the cell cycle in CRC cell lines. The results showed that oxaliplatin is a potent inhibitor of survivin, a member of the IAP (inhibitor of apoptosis) family. Since survivin is a key molecule involved in regulation of cell death (11), we examined the efficacy of oxaliplatin in combination with a putative apoptosis-inducing reagent, paclitaxel (taxol). The present study explains in part why oxaliplatin displays augmented effects of another class of chemoreagents and suggests the potential usefulness of various novel cancer therapies in combination with oxaliplatin.

MATERIALS AND METHODS

REAGENTS AND CELL LINES

Oxaliplatin was obtained from Yakult Co. (Tokyo, Japan), and stock solutions of 1 mg/ml in water were prepared and stored at −20°C. Paclitaxel was obtained from Bristol Myers Squibb Japan (Tokyo), and stock solutions of 10 mM in dimethylsulfoxide (DMSO) were prepared and stored at −20°C. Human CRC cell lines, DLD1, SW480 and HT29, were obtained from the American Type Culture Collection. They were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin in 5% CO₂ at 37°C.

WESTERN BLOT ANALYSIS

Western blot analysis was performed as described previously (12). The following antibodies, at appropriate concentrations, were applied on membranes after transfer of an SDS–polyacrylamide gel: anti-cdc2 mouse monoclonal antibody (POH1), anti-phospho-Bcl-2 (Ser70) rabbit polyclonal antibody, anti-Bcl-xl rabbit polyclonal antibody, anti-Bax rabbit polyclonal antibody (all from Cell Signaling Technology, Beverly, MA) and anti-survivin rabbit polyclonal antibody (Novus Biologicals, Littleton, CO). Equal loading of the protein samples was confirmed by parallel western blots for actin (anti-actin rabbit polyclonal antibody, Sigma-Aldrich, Sigma, St Louis, MO).

CELL CYCLE ANALYSIS

Flow cytometric analysis was performed as described previously (13). Data were acquired with a FACSort (Becton Dickinson Immunocytometry Systems, San Jose, CA). Analysis of the cell cycle was carried out using ModFIT software version 3.0 (Becton Dickinson Immunocytometry Systems).

ASSESSMENT OF APOPTOSIS BY ANNEXIN V STAINING

Quantification of apoptotic cells was performed using an Annexin V–FITC Apoptosis Detection Kit (BioVision, Mountain View, CA) according to the protocol recommended by the manufacturer. Briefly, cells were resuspended in 500 μl of binding buffer, and 5 μl of Annexin V–fluorescein isothiocyanate (FITC) and 5 μl of propidium iodide (PI) were added. Then cells were incubated at room temperature for 5 min in the dark, and analyzed for Annexin V–FITC binding by flow cytometry (FACSort) using an FITC signal detector (FL1) and a PI signal detector (FL2). The cells in the FITC-positive and PI-negative fraction were regarded as apoptotic cells. Each experiment was repeated three times and gave no discrepant results.

DETERMINATION OF CASPASE-3 ACTIVITY BY COLORIMETRIC ASSAY

Caspase-3 activity was determined using the Caspase-3 Colorimetric Assay Kit (BioVision). The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA. Briefly, the cells were homogenized in 50 μl of chilled cell lysis buffer, incubated for 10 min on ice and centrifuged at 10 000 g for 1 min at 4°C. Then, 50 μl of 2× reaction buffer containing 10 mM dithiothreitol (DTT) and 5 μl of 4 mM DEVD-pNA substrate (200 μM final concentration) were added to each of the samples (100 μg of proteins), and incubated for 2 h at 37°C. The absorbance of each well was measured at 405 nm wavelength by a microplate reader. Each experiment was repeated three times and gave no discrepant results.

TREATMENT SCHEDULE

The treatment schedule was determined with reference to previous reports on the schedule-dependent interaction between two cytotoxic reagents in vitro (14,15). Twenty-four hours after seeding (time 0), cells were incubated with one reagent for 24 h, followed by exchange of medium and incubation with another reagent for another 24 h. In single-reagent treatments, or simultaneous treatment, the cells were incubated for 24 h.

ISOBOLOGRAM ANALYSIS

The cells at exponential growth were seeded in triplicates in 96-well tissue culture plates at 2500 per well. After treatment with various concentrations of the reagents, viable cells were assessed by WST-1 assay (see below) at 72 h after initial administration of the reagent (time 0). Dose–response interactions between oxaliplatin and paclitaxel were evaluated by an isoologram. The theoretical basis of the isoologram method has been described in previous studies (16,17). Based on the dose–response curves of oxaliplatin and paclitaxel, three isoeffect curves were constructed (mode I, mode Ia and mode IIb). When the data points of the drug combination fell within the area surrounded by three lines (envelope of additivity), the combination was regarded as additive. When the data points fell to the left or the right of the envelope, the drugs were regarded as having a synergic or antagonistic effect, respectively.
Assessment of Cell Growth

For cultures in 6- or 12-well plates, the cell number was directly counted using a hemocytometer. For cultures in 96-well plates, the WST-1 assay was used. A 10 μl aliquot of WST-1 reagent [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2-disulfophenyl)-2H-tetrazolium, monosodium salt; Dojindo Laboratories Co., Kumamoto, Japan] was added to each well and incubated for 2 h at 37°C. Cell viability was determined based on mitochondrial conversion of WST-1 to yellowish formazan, indicative of viable cells. The absorbance at 450 nm was read with a microplate reader.

Assessment of Mitotic Catastrophe

Cells in triplicate cultures were treated with reagents and stained with hematoxylin solution. To assess mitotic catastrophe, 10 random fields were examined at high power magnification and the percentage of the cells undergoing mitotic catastrophe relative to the total number of cells was calculated. Enlarged and multinucleated cells were regarded as mitotic catastrophe (18). At least 700 cells were counted in each sample.

Statistical Analysis

Data are expressed as the mean ± SEM. Differences between values obtained in a population of cells treated with different experimental conditions were determined using the unpaired t-test. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed using the StatView 5.0 program (SAS Institute Inc., Cary, NC).

Results

IC50 Values of Colon Cancer Cell Lines with Treatment of Oxaliplatin

Cells were uniformly seeded (1 × 10^4/well) into 12-well dishes in triplicate. Twenty-four hours later, the culture medium was removed and replaced with 2 ml of fresh medium containing 0–50 μM oxaliplatin for 24 h, then incubated in drug-free medium for a further 48 h. The IC50 values of each cell line were 1.9 ± 0.7 μM for SW480, 10.3 ± 2.2 μM for DLD1 and 2.5 ± 0.4 μM for HT29.

Effects of Oxaliplatin on Apoptosis and Apoptosis-Related Protein Expression

The results are summarized in Table 1. Oxaliplatin significantly increased the percentage of apoptotic cells among the three CRC cell lines. The percentage of apoptotic cells was highest in DLD1 cells. A significant increase in caspase-3 activity was also noted in DLD1 cells, but not in other cells. Western blot analysis indicated that oxaliplatin did not essentially change the expression of Bax, an apoptosis-promoting protein, but apparently reduced the expression of apoptosis-inhibitory factors, phosphorylation of Bcl-2 and Bcl-xl as early as 24 h after treatment (Fig. 1). A clear decrease in the expression of phosphorylated Bcl-2 was evident at 48 h.

Effects of Oxaliplatin on Cell Cycle Distribution and Expression of Cdc2 Protein

Cell cycle analysis indicated that oxaliplatin treatment resulted in accumulation at S and/or G2–M in the three colon cancer cell lines (Fig. 2A). Oxaliplatin reduced the expression of cdc2, a positive regulator of the cell cycle acting at the G2–M phase, at 24 h in the three cancer cell lines (Fig. 2B). At 48 h, cdc2 levels recovered to some extent. Since cdc2 phosphorylates and stabilizes survivin (19,20), an inhibitor of apoptosis, we next examined the effect of oxaliplatin on survivin expression. In the three CRC cell lines, there was a marked decrease in survivin level at 24 and 48 h after treatment with oxaliplatin (Fig. 2B).

Effects of Combination Treatment of Oxaliplatin and Paclitaxel (Taxol)

In the combination assay, we used DLD1 and SW480 cell lines, which show relatively high sensitivity and low sensitivity to oxaliplatin-induced apoptosis, respectively.

Isobologram analysis. Isobologram analysis showed that the order of administration of the reagents carried great weight. Thus, administration of taxol followed by oxaliplatin, or simultaneous administration of the two reagents, resulted in additive or synergic effects on DLD1 and SW480 cells. In contrast, administration of oxaliplatin followed by taxol led to apparent antagonism in both cell lines (Fig. 3). Based on these findings, subsequent experiments were performed primarily in the

Table 1. Apoptosis and caspase-3 activity induced by oxaliplatin in colon cancer cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>24 h Induction of apoptosis (%)</th>
<th>48 h Induction of apoptosis (%)</th>
<th>Relative caspase-3 activity (fold induction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Oxaliplatin</td>
<td>Untreated</td>
</tr>
<tr>
<td>SW480</td>
<td>1.8 ± 0.5</td>
<td>3.2 ± 0.2*</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>DLD1</td>
<td>0.7 ± 0.1</td>
<td>13.6 ± 0.7**</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>HT29</td>
<td>1.8 ± 0.4</td>
<td>5.7 ± 0.4**</td>
<td>2.4 ± 0.9</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01.

IC50 values of each cell line were applied. SW480, 1.9 μM; DLD1, 10.3 μM; HT29, 2.5 μM.
Growth inhibitory effects. Compared with single-reagent treatment, the taxol–oxaliplatin sequence, but not the oxaliplatin–taxol sequence, displayed significantly greater growth inhibitory effects in both cell lines on day 6 (Fig. 4A). Similar results were obtained on day 3 (data not shown).

Apoptosis and caspase-3 activity. In DLD1 cells, the taxol–oxaliplatin sequence induced prominent apoptosis that reached ~32%. Morphological survey showed the presence of many shrunken apoptotic cells with a fragmented nucleus in the culture (Fig. 4B). There was a significant increase in apoptotic cells at 48 h in the taxol–oxaliplatin sequence, compared with single-reagent treatment (Fig. 4C). In contrast, the oxaliplatin–taxol sequence rather suppressed apoptosis relative to treatment by taxol alone. Caspase-3 activity was significantly increased by the taxol–oxaliplatin sequence, but was significantly decreased in the reverse sequence, compared with a single-reagent treatment, at 36 h. In SW480 cells, the taxol–oxaliplatin sequence caused a significant increase in apoptosis compared with single-reagent treatment, but the absolute value of apoptosis was rather low compared with that in DLD1 cells. Caspase-3 activity was not essentially changed amongst single-reagent groups and combination groups (Fig. 4C).

Mitotic catastrophe. During observation of cultures of SW480 cells treated with various agents, we found that giant multinucleated cells that are compatible with mitotic catastrophe were often present (Fig. 4A, arrows; Fig. 4B). Compared with a single-reagent treatment, the cells undergoing mitotic catastrophe with the taxol–oxaliplatin sequence increased up to 13% on day 3 and increased further up to 30% on day 6 (Fig. 4D). In DLD1 cells, on the other hand, mitotic catastrophe was only minimally present, with the highest incidence being <5% with the taxol–oxaliplatin sequence on day 6.

Survivin expression. Compared with non-treated cultures, taxol largely induced survivin expression in SW480 cells at 36 h. The taxol–oxaliplatin sequence, rather than the oxaliplatin–taxol sequence, decreased taxol-induced survivin expression (Fig. 5). At 48 h, similar results were obtained.

DISCUSSION
Recent studies on oxaliplatin using p53 wild-type HCT116 colon cancer cells have demonstrated that p53 and Bax genes play a role in cell apoptosis and that p53 and p21waf1/cip1 genes may be important in G1 arrest of the cell cycle (21,22). In the present study, we examined the effects of oxaliplatin in p53 mutant type cells, representing the major category in human sporadic CRC (23). We found that oxaliplatin decreased the levels of apoptosis inhibitory factors, phosphorylation of Bcl-2 and Bcl-xl in the p53 mutant CRC cells. In contrast to HCT116 cells treated with oxaliplatin (21), no accumulation of the Bax protein was observed. Apoptosis was indeed induced in the three p53 mutant CRC cell lines, and DLD1 cells in particular showed the highest incidence of apoptosis as well as enhanced caspase-3 activity. These findings are in part consistent with the previous report that antisense Bcl-xl enhanced the efficacy of oxaliplatin in HCT116 cells (22), and the present data additionally suggest the involvement of dysregulation of Bcl-2 in oxaliplatin-mediated apoptosis.

Cell cycle analysis in the present study indicated that oxaliplatin caused G2–M arrest and that such an effect is mediated at least in part by downregulation of cdc2. This possibility is supported further by the finding that HT29 cells showed the largest G2–M accumulation and had the most evident reduction in cdc2 level. In relation to cdc2, we focused on survivin, a representative constituent of the IAP family. Cdc2 maintains the stability of the survivin protein by phosphorylation on Thr34. Once cdc2 is inactivated, survivin is dephosphorylated, becomes unstable and easily undergoes degradation (19). This association between cdc2 and survivin was recently shown in tetra-O-methyl nordihydroguaiaretic acid-treated cells (20). In our study, a marked reduction in survivin level was
achieved by oxaliplatin in the CRC cell lines tested. Considered together, these findings suggest that downregulation of this anti-apoptotic protein may at least in part contribute to the induction of apoptosis by oxaliplatin. However, it is also possible that oxaliplatin may directly downregulate survivin expression, in partial cooperation with cdc2, or independently of cdc2.

In the past years, research on survivin provided cumulative information. Downregulation of survivin by oxaliplatin is of considerable importance because this anti-apoptotic protein is overexpressed in virtually every human cancer and high expression of survivin was shown to be associated with resistance to chemotherapy (24) and poor prognosis of carcinomas of the lung, breast, colon and esophagus (25–28). In vitro studies showed that inhibition of survivin restored or enhanced the cytotoxicity of chemoreagents (29,30), and animal studies showed a superb efficacy against xenografts using an adeno-viral strategy targeted to survivin (31,32). Recently, survivin

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**Figure 2.** Effects of oxaliplatin on cell cycle distribution and expression of cdc2 and survivin protein. (A) Flow cytometric analysis. Oxaliplatin treatment resulted in G2–M accumulation in all three colon cancer cell lines. The experiment was repeated twice and similar results were noted. Representative results are shown here. (B) Western blot for cdc2 and survivin expression. Oxaliplatin treatment decreased the expression of cdc2 at 24 h. Oxaliplatin treatment markedly decreased the survivin level at 24 and 48 h. Blots for actin served as loading controls. The IC50 concentration was applied for each cell line.
was found to be the downstream target of β-catenin/TCF transcription (33). Based on the above evidence, survivin is at present validated as a cancer therapeutic target (34) and we showed for the first time that oxaliplatin is a potent inhibitor of survivin. Studies showed that upregulation of survivin has been observed for several anti-cancer agents, such as taxol, cisplatin and doxorubicin, in accordance with the cytoprotective function of survivin (35,36). It is likely that carcinoma cells need the optimal amounts of survivin to create a balance with apoptotic signals induced by chemoreagents. On the other hand, downregulation of survivin appears to be characteristic of oxaliplatin because we are not aware of other chemoreagents that inhibit survivin.

Accordingly, we hypothesized that the effects of the oxaliplatin-mediated decrease in survivin might be more evident when apoptotic signals are activated by certain reagents. To explore this possibility, we employed paclitaxel (taxol), which is known as a potent inducer of apoptosis (37), with a relatively high level of survivin (Fig. 5). With this combination, we found that the order of drug administration was important to obtain efficient cytotoxicity, being consistent with recent reports by Tanaka et al. (38). This may be explained by the concept of cell cycle-mediated antagonism advocated by Shah et al. (39). Taxol induces cell death through mitotic arrest, with polymerization and stabilization of microtubules, thus it is necessary for the cells to proceed in the M phase to fulfill the maximum effects. However, if oxaliplatin is administered prior to taxol, downregulation of cdc2 may perturb entry of the cells into the M phase since cdc2 commits entry of eukaryotic cells into M phase and the commencement of mitosis (40).

Our results showed that administration of taxol followed by oxaliplatin induced a 3.9- to 6.3-fold strong growth inhibitory effect in DLD1 and SW480 cells compared with taxol alone. In DLD1 cells, the extensive apoptosis induced by taxol was further enhanced by subsequent administration of oxaliplatin (up to >30%) with upregulation of caspase-3 activity. We are not certain at present whether the augmented effect was exclusively due to downregulation of survivin by oxaliplatin, but there is at least evidence that inhibition of survivin by small interfering RNA significantly increased taxol-induced cell death (30). In SW480 cells, on the other hand, although an increase in apoptosis was noted due to taxol–oxaliplatin, the absolute value of apoptosis was as much as 10%, which is unlikely to account for the marked growth inhibition in combination therapy in this cell line. During the study, we became aware of the appearance of multinucleate giant cells in SW480 cultures following drug treatment (Fig. 4A, indicated by arrows, Fig. 4B). Such a morphological feature is compatible with mitotic catastrophe, a form of non-apoptotic cell death caused by aberrant mitosis (11). Besides the anti-apoptotic aspect, survivin localizes to various components of the mitotic

![Figure 3. Isobologram analysis. To determine the optimal order of drug administration, isobologram analyses were performed. Oxaliplatin and taxol were administrated at various concentrations as described in Materials and Methods. The range of concentration was as follows: oxaliplatin for DLD1, 0–40 μM; oxaliplatin for SW480, 0–5 μM; taxol for DLD1, 0–200 nM; taxol for SW480, 0–1000 nM. Administration of taxol followed by oxaliplatin, or simultaneous administration of the two reagents resulted in additive or synergistic effects in DLD1 and SW480 cell lines. In contrast, administration of oxaliplatin followed by taxol led to apparent antagonism in both cell lines.](image-url)
Fig. 4. Continued.
Oxaliplatin, a potent inhibitor of survivin

B

DLD1

SW480

L-OHP 10µM (Day 3)  Taxol 250 nM (Day 6)

C

DLD1

SW480

Fig. 4. Continued.
apparatus including centrosomes, mitotic spindle microtubules and midbodies, mainly to carry out a well-controlled distribution of chromatin and maintain the mitotic process in order to avoid cell division and induce mitotic catastrophe and eventually slow cell death (11). We found that taxol alone caused mitotic catastrophe in <10% of SW480 cells, being consistent with a report that taxol induced mitotic catastrophe in HeLa cells (42). Combination of taxol and oxaliplatin resulted in a marked increase in mitotic catastrophe after long exposure. This could explain the outstanding efficacy of combination therapy in SW480 cells. It was of interest that the taxol–oxaliplatin sequence, rather than the reverse sequence, decreased taxol-induced survivin expression.

Clinical studies have shown that monotherapy using oxaliplatin, or 5-FU and leucovorin (folic acid), is effective against colon cancer with an efficacy as great as 10 and 12–33%, respectively (43–45), while 5-FU, leucovorin plus oxaliplatin provided a much higher response rate of >50% against metastatic CRC (46). It is likely that 5-FU or irinotecan plus oxaliplatin may be of greater clinical efficacy than taxol plus oxaliplatin because taxol monotherapy is not effective in metastatic CRC thus far (47). Nevertheless, it is possible that combination therapy with taxol may be useful in patients who have acquired resistance to 5-FU- or CPT11-based chemotherapy. Indeed, a case report described 53% rectal cancer shrinkage following treatment with taxol and a second-generation platinum compound, carboplatin [(48), M. Ohi, personal communication].

Figure 4. Effects of oxaliplatin and taxol combination treatment on cell growth and apoptosis. (A) Growth inhibitory effects. Cells were seeded at 6 × 10^4 density in triplicate onto the 6-well tissue culture plates and treated with single or combination reagents, as described in Materials and Methods, at the IC_{50} concentrations (oxaliplatin, 2 μM for SW480 and 10 μM for DLD1 cells; taxol, 250 nM for SW480 and 50 nM for DLD1; these concentrations are within the clinically achievable range (51–53)). Six days after the initial treatment, the number of cells was counted. Separate plates containing glass coverslips were subject to hematoxylin staining and examined for morphological analysis. (a) Control, (b) oxaliplatin, (c) taxol, (d) taxol—oxaliplatin, (e) oxaliplatin—taxol. Cont., control; Tx., Taxol, *P < 0.01. Arrows indicate giant multinucleated cells compatible with mitotic catastrophe. Magnifications: ×100. (B) Typical morphological changes in apoptosis in cultures of DLD1 and mitotic catastrophe in cultures of SW480, when treated with the taxol–oxaliplatin sequence. Magnifications: ×400. (C) Apoptosis and caspase-3 assays. An apoptotic reaction was detected at a relatively early time (48 h). An increase in caspase-3 activity was detected at the earlier time point of 36 h. In DLD1 cells, there was a marked induction of apoptosis and caspase-3 activity in the taxol–oxaliplatin sequence, compared with single-reagent treatment. Cont., control; Tx., taxol. *P < 0.05, **P < 0.01. (D) Incidence of cells undergoing mitotic catastrophe. In cultures of SW480 cells, the cells undergoing mitotic catastrophe with treatment by the taxol–oxaliplatin sequence increased to 13% on day 3 and increased further up to 30% on day 6. Cont., control; Tx., taxol. *P < 0.05, **P < 0.01.
communication. In this context, the present results of the enhanced cytotoxicity of taxol plus oxaliplatin may have an important clinical implication. In other types of carcinomas, clinical studies already showed the efficacy of taxol–oxaliplatin therapy as 48% in ovarian cancer and 34.2% in non-small-cell lung cancer, with good tolerance (49,50). In addition to taxol, the present finding of marked inhibition of survivin by oxaliplatin warrants further investigation of the novel cancer therapy in combination with oxaliplatin.

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References


**Figure 5.** Survivin expression. Compared with non-treated cultures, taxol largely induced survivin expression in SW480 cells at 36 h. The taxol–oxaliplatin sequence, rather than the oxaliplatin–taxol sequence, decreased taxol-induced survivin expression. At 48 h, similar results were obtained.